THE OLIGODYNAMIC ACTION OF COPPER FOIL ON CERTAIN INTESTINAL ORGANISMS.

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Carl von Nägeli, probably the greatest botanist of the last century, being both a philosopher and a true scientist, passed away on May 10, 1891. Among his papers was found the manuscript of a paper entitled "Ueber oligodynamische Erscheinungen in lebenden Zellen," which, together with an added note by Cramer, was published by Schwendener several years after his death in Neue Denkschriften der schweizerischen naturforschenden Gesellschaft.[1]

This really remarkable paper, while it has attracted considerable attention, does not seem to have been given the credit in some quarters that its merits deserve. In the light of more recent biological studies it has proved to be one of the most important papers that was written by Nägeli, and illustrates both the fertility of his resources and the incisiveness of his genius.

In this paper Nägeli showed how exceedingly sensitive certain living plants are to very minute quantities of various metals. For forty years he had been studying the algæ, but it was not until some time in the '80's during an illness that he observed that if algæ were placed in distilled water they were killed. This he at first attributed to various causes, but found upon analysis of the water that it contained traces of copper, and later experiments showed that the copper, which had been dissolved by the water in its passage through the copper still, was the toxic agent. He then carried on a large number of experiments placing copper coins in distilled water, and even went so far as to calculate approximately the amount of copper which was dissolved.

[1] For example, in the English translation of Pfeffer's Physiology of Plants, Vol. II, page 260, it is stated that copper is poisonous to *Spirogyra* in the proportion of one part of copper to 1,000 million parts of water, an observation made by Nägeli in the paper referred to above, and yet no mention of this paper is made in the citation of literature, which would lead the reader to believe that one of the other investigators quoted deserved the credit for the discovery.

In these experiments Nägeli used 2-pfennig pieces, consisting of 95 parts of copper, 4 of tin and 1 of zinc. These were cleaned with sand, and twelve of them were placed in 12 liters of distilled water and allowed to remain for several days. The solution was evaporated, the residue dissolved in hydrochloric acid, and the copper precipitated as sulphide. This precipitate was dissolved in nitric acid and an excess of ammonia added, producing a blue solution. On comparing the intensity of color of this solution with that of other solutions containing known quantities of copper sulphate, Nägeli estimated that it contained 1.3 parts of copper to 100 million parts of water. He found that this solution was toxic to various species of *Spirogyra*, and a further experiment showed that if the solution were diluted ten times, that is, so that it contained 1.3 part of copper to 1,000 million parts of water, it would still kill *Spirogyra*.

Inasmuch as solutions containing such extremely minute quantities of copper were toxic to *Spirogyra*, Nägeli was inclined to believe that the toxic action was different from ordinary chemical poisoning. This view appeared to him to be strengthened by the fact that the effects produced in the cells were different from those produced by ordinary poisons or those resulting from the natural death of the organism.

It has been supposed by some later investigators[1] that Nägeli did not regard the copper as being in a state of solution, yet the experiments just described clearly show what his information was on this point, and in another part of his paper he distinctly states that he so regards it. The marvellous thing to him, as to us, was that such minute quantities of copper exerted toxic action, and at first he was inclined to believe that the effect produced was due to a new force "Isagität," and in his original manuscript he used the word "isagische" in describing it. But this term was later replaced

[1] On page 23 of his paper Nägeli says, "Die oligodynamischen Eigenschaften des Wassers lassen sich also in allen Fällen auf Stoffe, die im demselben gelöst sind, zuruckführen. Nun weicht aber das durch Metalle oligodynamische gewordene Wasser in seinem Verhalten wesentlich ab von anderen Lösungen. Eine Salz or Zuckerlösung verliert ihre eigenschaften nicht, wenn unlösliche Körper in dieselbe gelegt werden und sie erteilt den Wandungen des Gefässes nicht die Fähigkeit, reines Wasser wieder salsig or süss zu machen, währende analoge Erscheinungen bei den Kupferlösungen eintreten."

by that of "oligodynamische" derived apparently from two Greek words meaning the force within a small quantity of substance.[1]

There seems to be some confusion among recent writers as to the condition of the copper produced by placing copper foil in water, and it is customary to speak of the solution as being a solution of colloidal copper. While it has been customary since the classical experiments of Graham to apply the name colloid to those substances which in solution or suspension will not pass through animal membranes, still recent researches have shown as pointed out by Noyes[3] that there are two subclasses of colloidal mixtures, — the one having the characteristic properties of true solutions, that is, possessing osmotic pressure, diffusibility and usually a limited solubility at some temperature; the other being without these properties and being in the nature of macroscopic and microscopic suspensions. Considering the origin of the copper in solution it would properly belong to the class of colloidal suspensions, but it has none of the properties of this class of substances; and it differs fundamentally from the so-called colloidal solutions not only in origin but in that it possesses the property of permeating colloids, as the cell wall and the organized contents of the cell, thereby producing marked disturbances in the cell and thus resembling the crystalloids.

It is well known as stated by Copeland and Kahlenberg[2] (page 455) that "every metal in contact with water and air is subject to some change. It reacts with oxygen and carbonic acid dissolved in water, or with the water itself, to form oxides, hydroxides, carbonates, basic carbonates, or acids, which in greater or less degree pass into solution. When this chemical action is sufficient for the effect to become visible, the metal is tarnished or corroded; and even gold and platinum lose their lustre." Nägeli in his paper (page 24) says that a solution of copper manifesting oligodynamic properties results only when copper is placed in water containing oxygen and carbon dioxide; but so far no one has determined which compound, or compounds, of copper is formed under these conditions.

[1] As further indicating the meaning that Nägeli had in mind we quote from his paper (page 8) as follows: "Ich will nun, um eine bestimmte und feste Bezeichnung zu haben, die specifische Wirkungen des Giftes die chemischen, die jenigen der noch unbekannten Ursache, in dem ich dem Endresultat vorgreife, die *oligodynamischen* nennen."

While Nägeli's paper was incomplete he nevertheless had carried on a sufficient number of experiments to show that there is a marked difference in the sensitiveness of various species of *Spirogyra* to the oligodynamic action of copper. He found, for instance, that *Spirogyra orthospera* and other *Spirogyras* with lense-shaped nuclei were more resistant than the remaining species.[1] He also showed that there was a marked difference in the sensitiveness in some of these plants (*S. nitida*) at different times of the day.

While Nägeli confined his attention to studies on *Spirogyra* using copper coins[2] to produce the oligodynamic effects, other investigators since his time have carried on experiments with other organisms both plant and animal and have employed metallic copper and salts of copper as well. One of the most important of these researches is that by Israel and Klingmann.[4] These investigators studied the effects of copper on certain bacteria (as *Bacillus typhi, B. coli and Microspira comma*), as well as certain animal organisms (as *Amoeba, Difflugia oblonga, Hematococcus pluvialis, Paramecium Bursaria, Spirostomum ambiguum, Vorticella microstoma* and *Stylonychia mytilus*), and also on *Spirogyra*. They used copper foil and found that it had a marked toxic effect on all of the bacteria that they worked with, *B. typhi* being the most sensitive. They also found that by placing the solutions of copper containing the organisms in an incubator at a temperature of 35°–40° C. the toxic effects were manifested in 1 hour, whereas at the ordinary temperature similar effects were produced in two hours. In the case of the animal organisms, while the toxic effects were visible in most instances in but a few minutes, in *Vorticella* it required several hours for any toxic effects to be observed and it was found that *Stylonychia* might resist the action for 24 hours. These authors further found that water in which copper foil had been placed for 24 hours, could be diluted 100 times and still manifest oligodynamic effects on *Spirogyra*. In the latter instance

[1] Cramer made a similar observation with *S. setiformis* (?).

Israel and Klingmann (page 307) found that *S. Crassa* was killed in 15 minutes, *S. majuscula* in 30 minutes and *S. laxa* in 75 minutes.

[2] During his investigation Nägeli also discovered that minute quantities of other metals, as silver, lead, tin, iron and mercury manifested oligodynamic properties similar to copper.

the time required was 24 hours as against 8 minutes in the first instance.

While we have seen that solutions containing minute quantities of copper are exceedingly toxic to certain organisms, other investigators have shown that various plants not only withstand the influence of relatively large quantities of copper sulphate, but under certain conditions even appear to be benefited by its presence. With these various data before us we may say that while copper has a specific toxic action even in very minute quantities on certain organisms, it should be borne in mind that these same organisms manifest a specific sensitiveness towards copper and various other metals.[1]

These data are not only of great interest from a scientific point of view but in their practical application are of very great importance, and it was to be expected that pharmacologists would appreciate the important bearing of this line of investigation on their work. Cushny[5] among pharmacologists early recognized the value of these researches and the possibilities in their application in the prevention and treatment of disease. He states that while copper is comparatively harmless to man, yet it is exceedingly toxic to certain microörganisms and intestinal parasites. He says:

"Small quantities of copper may be taken for indefinite periods without any symptoms being induced, so that so far as man is concerned the general action of copper is unknown. . . . On the other hand, copper is a deadly poison to several of the lower plants. Thus, traces of copper added to the water in which they live, destroy some of the simpler algæ, and Nägeli asserts that 1 part

[1] While various explanations might be offered to show why such extremely minute quantities of copper in solution are sufficient to kill unicellular and filamentous algæ, bacteria, and unicellular animal organisms, and yet not affect multicellular plants and animals, whose cells are as delicate in structure as those of the unicellular organisms, it seems that this is in a measure due to the fact that in the latter the entire individual is comprised in a single cell, which performs all the vegetative as well as reproductive functions, and being entirely surrounded by the copper solution, all the life process are affected, there being no way for the organism to distribute the solution to other cells, and thus by a dilution minimize the toxic action of the copper. Or if some of the cells in the multicellular organism are destroyed or injured by exposure to the solution, others are formed to take their place from the more or less deep-seated meristematic cells. It is true that the idiosyncrasies in these organisms should also be borne in mind, some of them being more resistant than others.

of copper in 1,000,000,000 parts of water is sufficient to kill these plants. . . . Locke found that the traces of copper contained in water distilled in copper vessels were sufficient to destroy tubifex (one of the annelid worms) and tadpoles, while Bucholtz states that the development of bacteria is stopped by a solution of copper sulphate under 1 per cent. in strength. Copper thus seems to have a very powerful poisonous action on certain living forms and to be harmless to others, and the subject deserves further investigation. It is possible that it may prove to act prejudicially to some human parasites, and it is certainly less dangerous to man than many other remedies used as parasiticides and disinfectants."

It was not, however, until the publication of the bulletin on "A Method of Destroying or Preventing the Growth of Algæ and Certain Pathogenic Bacteria in Water Supplies" by Moore and Kellerman,[6] nearly a year ago, that the very great practical significance of work along these lines became apparent and general interest was aroused in the subject.

Since last fall we have been carrying on a series of experiments in the Microscopical Laboratory of the Philadelphia College of Pharmacy[1] with the view of testing the efficiency of metallic copper for destroying typhoid and colon bacilli in water. Some of the results obtained have already been published.[7]

In presenting the results of our experiments sufficient of the details will be given to show the manner in which the work was conducted.

In the first series of experiments here recorded water under three different conditions was employed: (*a*) Distilled water which was prepared from tap water by first treating it with potassium permanganate and then distilling it two or three times by means of apparatus constructed entirely of glass; (*b*) filtered tap water, prepared by means of a Berkefeld filter attached to a copper spigot; (*c*) tap water, collected after being allowed to run for five minutes, the spigot being the usual copper one. All of these were sterilized in an autoclave at 110° for 30 minutes.[2]

The cultures of typhoid and colon which were used were pure cultures developed in bouillon for 18 to 24 hours.

[1] I acknowledge my indebtedness to Mr. John R. Rippetoe for valuable assistance in carrying on the experiments recorded in this paper.

[2] In all of our work we found in the blank experiments that water which had been sterilized in an autoclave remained sterile.

To 200 c.c. of samples of water prepared as stated, and contained in sterile Erlenmeyer flasks, were added two 3-mm. loops of the fresh bouillon cultures of typhoid and colon bacilli respectively. Counting the duplicate experiments provided for, we thus had a series of 12 flasks, 6 of them containing typhoid bacilli, and 6 colon bacilli.

For determining the number of organisms, 1 c.c. of the respective solutions was transferred directly to a Petri dish by means of a sterile 1-c.c. pipet, and to this was added 10 c.c. of Heyden's nutrient agar, which had been kept at a temperature of 40° C. for some time. Three separate plates of the water in each of the 12 flasks was made immediately upon the addition of the cultures, and both the plates and the flasks were kept at a temperature of 35°

TABLE I.—EXPERIMENTS WITH *Bacillus coli.*

Plates Made.	Water Without Copper Foil.			Water With Copper Foil.		
	Triple Distilled Water	Filtered Tap Water.	Tap Water.	Triple Distilled Water.	Filtered Water.	Tap Water.
At time of adding culture.	7,746	11,246	8,283	8,866	4,410	6,790
At end of 4 hours.	7,655	5,075	7,665	No organisms.	No organisms.	No organisms.
At end of 8 hours.	7,735	3,115	7,000	"	"	"
At end of 24 hours.	1,000,000	1,000,000	1,500,000	"	"	"
At end of 48 hours.	1,200,000	1,600,000	2,000,000	"	"	"
At end of 6 days.	1,200,000	1,000,000	1,200,000	"	"	"
At end of 14 days.	1,060,000	910,000	2,245,000	....	....	....
At end of 21 days.	700,000	462,000	650,000	....	....	....
At end of 28 days.	700,600	462,446	649,666	....	....	....
At end of 53 days.	602,000	456,000	693,000	....	....	....
At end of 60 days.	583,200	421,000	687,333	....	....	....
At end of 83 days.	215,600	128,766	206,950	....	....	....
At end of 90 days.	208,133	48,433	147,000	....	....	....
At end of 130[1] days.	289,333	146,543	225,400	....	....	....

[1] The nutrient medium used in the plates made at the end of 130 days was agar having an acidity of 0.5 percent.

C. to 37° C. To six of the flasks were then added strips of copper foil about 15 mm. wide and 18 cm. long, these being corrugated in such a manner that the entire surface was exposed to the water.

Plates were made from all the 12 flasks at the end of 4 hours and 8 hours, and 1 day, 2 days, and 6 days, even in the cases where no organisms remained, and in the cases in which they continued to develop, also at the end of 14, 21, 28, 53, 60, 83, 90, 120, 130 and 134 days. The results are given in the accompanying tables.

TABLE II.—EXPERIMENTS WITH *Bacillus typhosus*.[1]

Plates Made.	Water Without Copper Foil.			Water With Copper Coil.		
	Triple Distilled Water.	Filtered Tap Water.	Tap Water.	Triple Distilled Water.	Filtered Water.	Tap Water.
At time of adding culture.	3,740	4,750	3,675	3,986	127	1,400
At end of 4 hours.	2,835	No organisms.	3,815	No organisms.	No organisms.	No organisms.
" " 8 "	3,850	"	1,995	"	"	"
" " 24 "	3,750	"	1,435	"	"	"
" " 48 "	3,815	"	1,540	"	"	"
" " 6 days.	1,850	"	....	"	"	"
" " 14 "	16,380	"	3,920	....	....	....
" " 21 "	39,690	"	65,500	....	....	....
" " 28 "	153,600	"	221,867	....	....	....
" " 60[2] days.	295,866	"	961,800	....	....	....
" " 90 "	239,400	"	346,500	....	....	....
" " 120 "	78,750	"	9,156	....	....	....
" " 134 "	34,440	"	7,875	....	....	....

[1] Bouillon cultures of the different samples of water, at the end of 60 days, gave with Widal's test the characteristic behavior of typhoid organisms. After 60 days the organisms were found to be very long and more or less filamentous and did not respond to Widal's test. I am indebted to Dr. Herman B. Allyn, Philadelphia, for specimens of typhoid blood.

It is seen in the foregoing tables that in all the flasks to which copper foil had been added all of the organisms were destroyed in less than four hours, and furthermore the solutions remained sterile as shown by plates made for a number days thereafter.

I may say that every single experiment which we have conducted, not only those given in the foregoing tables, but all others, shows that copper foil is exceedingly toxic to colon and typhoid bacilli, particularly the latter.

It will be seen further that in the filtered water, to which no copper foil had been added, the typhoid organisms did not develop as was the case with the tap water and distilled water, although there was a larger number of organisms to begin with; while the colon bacilli multiplied considerably in the filtered water still there was a very marked inhibiting action. At first I was inclined to attribute this diminution in the number of the organisms to minute traces of copper in the flasks, but subsequent experiments showed that this was not the case. I was, then, inclined to attribute these rather anomalous results to the presence of extremely small quantities of copper dissolved by the water in its necessarily slow passage through the copper spigot to which the filter was attached.

In order to test further the validity of this assumption another series of experiments was conducted using (*a*) tap water, (*b*) water filtered through a stone filter,[1] and (*c*) water filtered through a Berkefeld filter. The water in each case was sterilized in an autoclave at a temperature of 110° C. for 30 minutes, and 18- to 24-hour cultures of typhoid and colon bacilli were respectively added to the samples of water at the ordinary temperature. The results are summarized as follows:

TABLE III.—EXPERIMENTS WITH *Bacillus coli* AND *B. typhi* IN FILTERED WATER.

Plates Made.	*Bacillus coli.*			*Bacillus typhi.*		
	Tap Water.	Stone Filtered Water.	Berkefeld Filtered Water.	Tap Water.	Stone Filtered Water.	Berkefeld Filtered Water.
At time of adding culture.	5,040	10,611	10,269	7,875	1,512	1,764
At end of 2 hours.	6,426	18,270	6,600	5,040	....	....
" " 4 "	8,505	24,570	5,500	2,714	2,520	No organisms.
" " 6 "	6,930	28,350	2,646	250	....	"
" " 8 "	16,065	77,175	3,654	150	2,930	"
" " 24 "	315,000	630,000	150,000	38	3,829	"
" " 48 "	630,000	1,000,000	200,000	39	1,820	"
" " 7 days.	....	....	....	....	9,000	"
" " 14 "	1,289,333	1,505,700	599,333	80,770	43	"
" " 21 "	....	....	....	....	No organisms.	"
" " 30 "	900,000	1,260,000	94,500	No organisms.	"	"
" " 60 "	730,800	945,000	149,331	"	"	"

[1] In the preliminary experiments with samples of water that had been filtered through a stone filter or a Jewett filter, it was found that there was a similar inhibiting action on the organisms to that of water from the Berkefeld filter. This action was supposed to be due to the influence of the copper in the spigot attached to the receiver of the filter, and was overcome by removing the spigot and using a rubber stopper fitted with a glass tube.

It is seen from the foregoing table that while we began with approximately 5,000 organisms of colon bacilli to the cubic centimeter in the case of the tap water, there were over 700,000 at the end of sixty days; and that in the case of the stone filtered water where the initial number of organisms was about 10,000 they increased on an average similar to those in the tap water. In the case of the water from the Berkefeld filter, however, beginning with 10,000 organisms to the cubic centimeter, there was a rapid diminution of the organisms, so that but about 2,500, or about 25 per cent. of the organisms persisted at the end of six hours, and while they continued to multiply after this still the number was considerably less than in either the tap water or stone filtered water, showing that with Berkefeld filtered water there is some agency which inhibits the growth of the colon bacilli. This we concluded to be due to the copper dissolved from the spigot to which the filter was attached, as already suggested.

In the experiments with the typhoid organisms it was found that they multiplied in number in both the tap water and stone filtered water persisting for fourteen days, after which they disappeared, as was also the case in some other experiments; but in the case of Berkefeld filtered water they entirely disappeared within four hours, which was also the case in three other experiments not here recorded. It may also be stated that it was not unusual to observe in the case of both tap and stone-filtered water, where cultures of the typhoid bacillus were used, that if the organisms persisted until the fourteenth day, they would multiply enormously after that as shown for tap water and distilled water in Table II.

In an investigation of this kind many lines of experiment are suggested, and it was thought desirable to carry on another series of experiments with a view of testing the toxicity of solutions in which metallic copper had been allowed to remain for varying lengths of time. In these experiments sterilized distilled water and stone filtered water were used. To 600 cc. of water in a graduate 8 strips of copper foil 15×130 mm. were added. The graduate was agitated continuously and 100 cc. of the solution were removed at the end of 1, 5, 10, 20 and 30 minutes. The respective solutions were placed in Erlenmeyer flasks and sterilized in an autoclave at 110° C. for 30 minutes. To these were added 18- to 24-hour cultures of typhoid bacilli, and plates made with results as indicated in the two following tables:

TABLE IV.—EXPERIMENTS WITH *Bacillus typhi* IN DISTILLED WATER IN CONTACT WITH COPPER FOIL FOR VARYING LENGTHS OF TIME.

Plates Made.	Water *without* Copper.	Water in Contact *with* Copper for				
		1 minute.	5 minutes.	10 minutes.	20 minutes	30 minutes.
At time of adding culture	3,451	7,119	7,420	6,791	8,631	12,726
At end of 2 hours.	5,292	No organisms.	No organisms.	2,139	3,150	6,188
" 4 "	6,489	"	"	No organisms.	25	420
" 6 "	5,950	"	"	"	3	35
" 8 "	4,410	"	"	"	No organisms.	12
" 24 "	6,489	"	"	"	"	No organisms.
" 48 "	8,410	"	"	"	"	"
" 3 days.	11,466	"	"	"	"	"
" 4 "	7,560	"	"	"	"	"
" 7 "	2,898	"	"	"	"	"

TABLE V.—EXPERIMENTS WITH *Bacillus typhi* IN STONE FILTERED WATER IN CONTACT WITH COPPER FOIL FOR VARYING LENGTHS OF TIME.

Plates Made.	Water *without* Copper.	Water in Contact *with* Copper for				
		1 minute.	5 minutes.	10 minutes.	20 minutes.	30 minutes.
At time of adding culture.	5,050	4,725	7,221	6,111	13,482	11,403
At end of 2 hours.	4,599	4,977	6,615	7,056	16,000	11,088
" 4 "	6,300	5,859	3,906	6,339	14,000	15,482
" 6 "	7,119	6,300	4,250	5,418	8,946	5,574
" 8 "	4,914	8,064	5,481	5,645	7,951	5,624
" 24 "	10,710	3,213	2,205	1,925	4,410	....
" 48 "	10,700	1,155	142	104	790	3
" 3 days.	11,277	152	No organisms.	No organisms.	123	No organisms.
" 4 "	10,395	No organisms.	"	"	No organisms.	"
" 7 "	7,899	"	"	"	"	"

Table IV shows that in the experiments made with distilled water, the mere contact of the copper foil with the water for from 1 to 5 minutes imparted to it sufficient toxicity, or oligodynamic property, to kill the typhoid organisms placed in the solution within two hours, when the organisms did not exceed approximately 7,000 to the cubic centimeter, or 700,000 to the entire solution. Where the number of organisms in the solution exceeded this number approximately three-tenths of 1 per cent. persisted four to eight hours longer.

In the case of stone filtered water (Table V) a longer time was required to affect the organisms. This is probably accounted for by the fact that the water contained other substances which modified the action of the copper either precipitating it, absorbing it, or even adsorbing it, and thus weakening the solution.[1]

As showing the influence of a material which would be in the nature of a food to the organisms and which at the same time would have a tendency to inhibit the oligodynamic action of the copper solution, the following experiments were conducted using filtered water: (*a*) Berkefeld filtered water; (*b*) stone filtered water. In both series of experiments 1 cc. of nutrient bouillon was added to 200 cc. of water, which was then sterilized in the autoclave, and the typhoid organisms added after cooling.

TABLE VI.—EXPERIMENTS WITH *Bacillus typhi* IN FILTERED WATER CONTAINING BOUILLON.

Plates Made.	Berkefeld Filtered Water.		Stone Filtered Water.	
	Without Bouillon.	*With* Bouillon.	*Without* Bouillon.	*With* Bouillon.
At time of adding culture.	7,245	1,296	14,044	2,151
At end of 4 hours.	550	9	11,907	1,323
" 8 "	No organisms.	5	7,560	4,820
" 24 "	"	17	No organisms.	3,000,000
" 48 "	"	2,500,000	"	4,500,000
" 7 days.	"	7,000,000	....	2,255,000
" 14 "	"	....	11,109	....
" 30 "	"	....	6,466	....
" 60 "				

In the case of the Berkefeld-filtered water it is seen that there was no growth in the flasks to which bouillon had not been added, after four hours; and while there was a diminution of the number

[1] Nägeli found (p. 13 of his paper) that the oligodynamic action of a copper solution could be lessened by the introduction of the following substances: Sulphur (either roll or flowers), carbon (either graphite or soot), coke, coal, peat, black oxide of manganese, starch, cellulose (either as Swedish filter paper, or cotton, linen or wood fiber), silk, wool, stearic acid, paraffin, gum, dextrin, egg albumin and glue.

True and Oglevee [8] have studied the influence of insoluble substances on the toxic action of poisons and have confirmed several of Nägeli's observations.

Moore and Kellerman have shown in their recent bulletin the relative decrease of toxicity of copper sulphate solutions depending on the amount of organic matter present, the amount of carbon dioxide in solution or the temporary hardness of the water.

of organisms in those solutions containing bouillon between the first 4 and 24 hours, there was after this a marked increase in growth. This increase in development would appear to begin after the last inhibiting traces of copper are removed, either by precipitation in the organisms or by the bouillon.

Other experiments which we conducted showed that there was a difference in the persistence of the typhoid organisms depending upon whether the cultures added to the water were 24-hour or 14-day bouillon cultures, as seen in the following table.

TABLE VII.—EXPERIMENTS WITH CULTURES OF *Bacillus typhi* OF DIFFERENT AGES.

Plates Made.	Tap Water.		Berkefeld Filtered Water.	
	24-Hour Cultures.	14-Day Cultures.	24-Hour Cultures.	14-Day Cultures.
At time of adding cultures.	3,058	1,050	1,983	952
At end of 4 hours.	682	1,105	40	574
" 8 "	440	604	No organisms.	215
" 24 "	137	217	"	106
" 48 "	63	179	"	150
" 7 days.	No organisms.	49	"	35

The figures in Table VII, show that the older cultures of the typhoid organisms were most resistant in the tap water, and that they survive over 7 days in Berkefeld-filtered water, the 24-hour cultures usually being destroyed in about 4 hours.

TABLE VIII.—EXPERIMENTS ON TAP WATER WITH COPPER FOIL AND COPPER SULPHATE.

Plates Made.	Tap Water *without* Copper Foil or Copper Sulphate.		Berkefeld Filtered Water.	Tap Water with Copper Foil.	Tap Water with Copper Sulphate.	
					1 Part to 100,000	1 Part to 1,000,000
At time of adding copper foil or copper sulphate.				39,000	8,233	8,233
On drawing tap water or before filtering.	39,000	8,233	46,800			
After filtering.			666			
At end of 2 hours.	32,666	9,500	....	35,666	300	1,833
" " 4 "	21,300	7,766	....	29,266	66	1,300
" " 6 "	40,900	10,200	....	20,516	200	2,233
" " 8 "	41,000	13,333	....	9,866	300	1,166
" " 3 days.	68,933	102,200	61,466	609,900	3,633	112,300
" " 6 "	....	87,100	500,200	....	185,000	97,150
" " 10 "	27,133	111,000	395,300	33,600	211,760	134,000
" " 13 "	....	....	343,700	....	....	....

At the beginning of our investigation a number of experiments were made with a view of testing the comparative efficiency of both copper foil and copper sulphate in destroying the organisms in tap water, and it is thought that the results obtained are of sufficient interest to present at this time.

It is observed that in the case of the Berkefeld filtered water, 99 per cent. of the original number of organisms were removed by the process of filtration. When copper foil was introduced into the water about 75 per cent. of the organisms were destroyed in 8 hours, although in other experiments where larger quantities of water were used from 85 to 97 per cent. of the organisms were destroyed. When copper sulphate was added to the tap water, so that there was 1 part to 100,000 of water, 97 per cent. of the organisms was destroyed in 8 hours. When the strength was reduced so that there was 1 part of copper sulphate to 1,000,000 parts of water, there was a reduction of 86 per cent.

Owing to the sensitiveness of typhoid and colon bacilli to the influence of copper, as previously shown, it may be inferred that they would have been included in the 75 to 97 per cent. of the organisms destroyed.

CONCLUSIONS.

From the experiments thus far conducted as well as the results obtained by other writers, the following conclusions may be drawn :

1. Certain intestinal bacteria like colon and typhoid are completely destroyed by placing clean copper foil in water containing them, or by adding the organisms to water previously in contact with copper foil.

2. The toxicity of water in which either copper coins or copper foil has been added is probably due to a solution of some salt of copper, as first suggested by Nägeli.

3. The copper is probably in the form of a crystalloid rather than that of a colloid, as it has the property of permeating the cell walls and organized cell contents of both animals and plants, thereby producing the toxic effects.

4. While the effects produced by the oligodynamic action of copper are apparently different from those of true chemical poisons, the difference is probably in degree only and not in kind.

5. Certain lower organisms including both plants and animals possess a specific sensitiveness to minute quantities of copper, and

it has been shown that they are not restored on transferring them to water free from oligodynamic properties.

6. Oligodynamic solutions of copper are obtained by adding either copper coins, copper foil or salts of copper to water; when copper foil is used, sufficient copper is dissolved by the distilled water in 1 to 5 minutes to kill the typhoid organisms within two hours.

7. A solution of copper may lose its toxicity by the precipitation of the copper as an insoluble salt or compound; by its absorption by organic substances; or by adsorption by insoluble substances.

8. The oligodynamic action of the copper is dependent upon temperature as first pointed out by Israel and Klingmann.

9. The effects of oligodynamic copper in the purification of drinking water are in a quantitative sense much like those of filtration, only the organisms removed, like *B. typhi* and *B. coli* are completely destroyed.

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